

# Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans<sup>1-3</sup>

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## ABSTRACT

**Background:** Carotenoids and tocopherols, known to be efficient antioxidants and capable of scavenging reactive oxygen species generated during photooxidative stress, may protect the skin from ultraviolet light-induced erythema.  $\beta$ -Carotene is widely used as an oral sun protectant but studies on its protective effects are scarce.

**Objective:** The objective of this study was to investigate the protective effects of oral supplementation with carotenoids and a combination of carotenoids and vitamin E against the development of erythema in humans.

**Design:** A carotenoid supplement (25 mg total carotenoids/d) and a combination of the carotenoid supplement and vitamin E [335 mg (500 IU) *RRR*- $\alpha$ -tocopherol/d] were given for 12 wk to healthy volunteers. Erythema was induced by illumination with a blue-light solar simulator. Serum  $\beta$ -carotene and  $\alpha$ -tocopherol concentrations and skin carotenoid levels were assessed by HPLC and reflection photometry.

**Results:** Serum  $\beta$ -carotene and  $\alpha$ -tocopherol concentrations increased with supplementation. Erythema on dorsal skin (back) was significantly diminished ( $P < 0.01$ ) after week 8, and erythema suppression was greater with the combination of carotenoids and vitamin E than with carotenoids alone.

**Conclusion:** The antioxidants used in this study provided protection against erythema in humans and may be useful for diminishing sensitivity to ultraviolet light. *Am J Clin Nutr* 2000;71:795–8.

**KEY WORDS** Carotenoids, tocopherol, sunburn, skin, erythema, healthy adults, ultraviolet light

## INTRODUCTION

$\beta$ -Carotene supplements are widely used as so-called oral sun protectants. However, studies on the protective effect of oral  $\beta$ -carotene supplements against skin responses to sun exposure are scarce. The protective effects are thought to be related to the antioxidant properties of the carotenoid. With ultraviolet (UV) irradiation, skin is exposed to photooxidative damage induced by the formation of reactive oxygen species such as singlet molecular oxygen ( $^1\text{O}_2$ ), superoxide radical anion ( $\text{O}_2^{\cdot-}$ ), and peroxyl radicals (1). Photooxidative damage affects cellular lipids, proteins, and DNA and is considered to be involved in the pathobiochemistry of erythema, premature aging of the skin,

photodermatoses, and skin cancer (2).  $\beta$ -Carotene, other carotenoids, and tocopherols are efficient scavengers of reactive oxygen species (3).

In vitro studies showed that carotenoids are among the most effective naturally occurring quenchers of  $^1\text{O}_2$ , with bimolecular rate constants in the range of  $1 \times 10^9$  to  $1 \times 10^{10} \text{ mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$  (4–6). In addition, carotenoids interact with peroxyl radicals, thus inhibiting lipid peroxidation (7, 8).  $\alpha$ -Tocopherol is less active as a quencher of  $^1\text{O}_2$  but occurs at a correspondingly higher plasma concentration (9); it is among the most effective lipid-soluble inhibitors of lipid peroxidation in human blood (10). Tocopherol and carotenoids interact with each other in the scavenging process (11, 12), and a synergism was found in multilamellar liposomal systems in lipid peroxidation induced by 2,2'-azobis(2,4-dimethylvaleronitrile) (13).

Several animal studies and in vitro experiments provided evidence that carotenoids and tocopherols prevent UV light-induced skin lesions and protect against skin cancer. Several human studies showed that plasma and skin carotenoid concentrations decrease with UV irradiation; lycopene is lost preferentially over other carotenoids (14, 15). Thus, beneficial effects of supplementation have been postulated.

Garmyn et al (16) found no protective effect of  $\beta$ -carotene given to subjects for 23 d at a dosage of 90 mg/d, although plasma and skin  $\beta$ -carotene concentrations were higher than control values. The subjects were exposed to a dose of solar simulated light that was 3 times the individually determined minimal erythema dose (MED), but there was no clinically or histologically detectable protection during  $\beta$ -carotene supple-

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mentation. In contrast, a protective effect of  $\beta$ -carotene was observed by Gollnick et al (17), who found that the development of erythema was lower in a group of volunteers who had received 30 mg  $\beta$ -carotene/d for 10 wk before exposure than in control subjects.

Recently, we showed that serum and skin  $\beta$ -carotene concentrations increased with supplementation with carotenoids derived from the alga *Dunaliella salina* for 12 wk (18). The present study was performed to investigate potential protective effects of carotenoids and a combination of carotenoids and *RRR*- $\alpha$ -tocopherol against erythema induced by UV light exposure.

## SUBJECTS AND METHODS

### Study design

Twenty healthy subjects (6 men and 14 women) aged 20–57 y with skin type I or II took part in the study and were randomly assigned to 2 groups. Skin type was graded according to skin color, hair and eye color, and history of sensitivity to sun exposure (19). The criteria for skin type I are fair, white skin; red or blonde hair; green or blue eyes; extreme sensitivity to sun exposure; and absence of tanning. The criteria for skin type II are white skin, blonde or light-brown hair, blue eyes, sensitivity to sun exposure, and minimal tanning. Only one participant with skin type I was assigned to each group. Subjects who smoked >3 cigarettes/d were not included in the study. Written, informed consent was obtained from each participant. The study design was approved by the ethical committee of the University of Witten.

An extract of *Dunaliella salina* (Betatene; Betatene Ltd, Melbourne) was used as the carotenoid source. This extract is used widely as a component of supplements with a high bioavailability of  $\beta$ -carotene. The supplement contained  $\approx 20\%$  of a carotenoid mixture (mainly  $\beta$ -carotene) in soybean oil; the supplement also contained low amounts of algal sterols and algal hydrocarbons (3–5%). One capsule contained 25 mg carotenoids comprising 13.0 mg *all-trans*- $\beta$ -carotene, 10.5 mg 9-*cis*  $\beta$ -carotene, 0.3 mg other *cis* isomers of  $\beta$ -carotene, 0.75 mg  $\alpha$ -carotene, 0.18 mg cryptoxanthin, 0.15 mg zeaxanthin, and 0.12 mg lutein.  $\alpha$ -Tocopherol supplements were obtained from Hermes (Munich, Germany) and contained 335 mg (500 IU) *RRR*- $\alpha$ -tocopherol/capsule.

In group 1, the carotenoid supplement was given daily for 12 wk; capsules were taken with the main meal. In group 2, the carotenoid supplement and the  $\alpha$ -tocopherol supplement were given daily for 12 wk; capsules were taken with the main meal. The diet was not standardized during the study but the participants were advised not to change their dietary habits. No further vitamin or carotenoid supplementation was allowed. Compliance was checked by questionnaire and by analyses of carotenoid and tocopherol serum concentrations. The response of unprotected skin before supplementation was used as a control; thus, groups 1 and 2 served as their own controls.

### Blood collection and analysis

Blood samples were collected on day 0 and after 4, 8, and 12 wk of supplementation. An additional blood sample was obtained 2 wk after cessation of supplementation. Serum was prepared from the blood samples and stored at  $-20^{\circ}\text{C}$  until analyzed. The analyses of  $\beta$ -carotene in serum were performed by

HPLC, as described previously (20).  $\alpha$ -Tocopherol concentrations were measured in the same samples as those used for the  $\beta$ -carotene analyses; an additional detector was switched in line and set to a detection wavelength of 292 nm.  $\alpha$ -Tocopherol was measured by using the external-standard method.

### Reflection spectroscopy

Skin carotenoid levels were measured by reflection spectroscopy at the blood sampling times. The details of the data analysis were described previously (18). This method measures  $\beta$ -carotene and all carotenoids in skin with identical UV-VIS spectra (eg, zeaxanthin);  $\alpha$ -Carotene, lutein,  $\beta$ -cryptoxanthin, and lycopene are not included. The skin carotenoid level was measured at the inside of the arm because this area is easily accessible to reflection photometry and is only moderately exposed to natural sunlight. In addition, the carotenoid level on the inside surface of the arm correlates well with serum  $\beta$ -carotene concentrations (18). Reflection spectra were collected noninvasively between 350 and 850 nm with a Multiscan OS 20 spectrophotometer (MBR GmbH, Herdecke, Germany) coupled with an all-silica fiberoptic reflectance bundle (Top Sensor Systems, Eerbeek, NL). Generally, an average spectrum consisted of 8 scans; each scan was completed within 124 ms. The spectral resolution was  $\approx 1.2$  nm; spectra were measured against a white reference standard (titanium oxide). A 5-W (5 J/s) halogen lamp (MBR GmbH) was used for tissue illumination. Under these conditions, the increase in skin surface temperature was  $<0.5^{\circ}\text{C}$ .

### Induction of erythema and measurement of skin color

Irradiation with UV light to induce erythema was applied only to dorsal skin (back, scapular region) by using a blue-light solar simulator (Hönle, Munich, Germany); different skin areas were irradiated at each time point. An MED was determined for each subject before the study. To measure responses of unprotected skin (controls), selected areas were exposed to 1, 1.25, 1.5, and 1.95 times the MED and skin color was measured before and 24 h after exposure (maximum erythema). During supplementation, skin was exposed to 1 MED at week 0, 1.25 MED at week 4, 1.5 MED at week 8, and 1.95 MED at week 12; additional UV irradiation (1.25 MED) was applied 2 wk after cessation of supplementation. At each time point, skin color was measured before and 24 h after irradiation.

Skin color was evaluated by chromatometry (Chromatometer CR 200; Minolta, Ahrensburg, Germany) with the 3-dimensional color system (*L*, *a*, and *b* values). *L* values are an indicator of lightness of skin and *b* values (blue-yellow axis) are an indicator of pigmentation; *a* values (red-green axis) are a measure of erythema formation and  $\Delta a$  values (*a* value 24 h after irradiation minus *a* value before irradiation) were used to measure skin responses to UV irradiation. Two subjects in group 1 and one in group 2 did not respond to increases in MED in the control experiment; because technical problems could not be ruled out, these subjects were excluded from the study.

### Statistical analyses

Statistical analyses were performed with EXCEL 5.0 (Microsoft Corp, Unterschleissheim, Germany); a two-tailed paired Student's *t* test was used. Comparisons were made within groups, between treatment and control (same subjects), and between groups. Differences were analyzed at each time point. All data are presented as means  $\pm$  SDs.

## RESULTS

### Serum and skin carotenoid concentrations

Supplementation with carotenoids (25 mg/d) or carotenoids plus vitamin E (335 mg *RRR*- $\alpha$ -tocopherol/d) for 12 wk was associated with elevated  $\beta$ -carotene concentrations in serum and carotenoid levels in skin (Table 1). After 12 wk of supplementation, a slight yellowing of the skin was observed in both groups; the palms of the hands and the facial skin were especially affected.

In group 1, the mean serum  $\beta$ -carotene concentration increased from 0.54  $\mu$ mol/L on day 0 to 2.92  $\mu$ mol/L after 12 wk of supplementation. The mean skin (inside of the arm) carotenoid level increased from 0.12 to 0.32 nmol/g. Two weeks after cessation of supplementation, the mean serum  $\beta$ -carotene concentration decreased to 1.67  $\mu$ mol/L and skin carotenoids decreased to 0.21 nmol/g. Serum vitamin E concentrations remained almost constant in this group (Table 1) and were quite high compared with data in the literature but still within the normal range.

In group 2, which received the same amount of carotenoids but an additional 335 mg *RRR*- $\alpha$ -tocopherol/d, the mean serum  $\beta$ -carotene concentration increased from 0.69  $\mu$ mol/L at baseline to 2.59  $\mu$ mol/L at week 12; the mean skin carotenoid level increased from 0.17 to 0.24 nmol/g (Table 1). Two weeks after cessation of supplementation, the mean serum  $\beta$ -carotene concentration decreased to 1.61  $\mu$ mol/L. No decrease in skin carotenoid levels was detectable. In this group, a substantial increase in serum vitamin E was observed. The mean serum vitamin E concentration increased from 42.0  $\mu$ mol/L on day 0 to 75.6  $\mu$ mol/L after 12 wk of treatment; after cessation of supplementation, the mean tocopherol concentration decreased to 47.9  $\mu$ mol/L (week 14). In contrast with vitamin E concentrations, mean serum and skin  $\beta$ -carotene concentrations were not significantly different between groups. Thus, there was no obvious influence of high vitamin E intake on the distribution of  $\beta$ -carotene into skin.

### Erythema assessment

Before supplementation all subjects were exposed to UV light at 1.0, 1.25, 1.5, and 1.95 times their individual MEDs. Directly after exposure and 24 h after irradiation, erythema was assessed (*a* value) and  $\Delta a$  values were calculated as a measure of unpro-

tected reaction (control values are shown in Table 1). The  $\Delta a$  values obtained from these exposures were used as controls; there was no significant difference in control values between groups. During supplementation with carotenoids or carotenoids plus vitamin E, the volunteers were again exposed to UV irradiation; exposure was 1.0 MED on day 0, 1.25 MED at week 4, 1.5 MED at week 8, and 1.95 MED at week 12.  $\Delta a$  values were calculated again (Table 1) and compared with the  $\Delta a$  values from the control experiment.

In both groups, the  $\Delta a$  values determined during supplementation were lower than the control values, indicating a protective effect. A significant difference was observed in group 1 between weeks 8 and 12 at 1.5 and 1.95 times the MED, respectively. In group 2, the differences between  $\Delta a$  values determined in the control experiment and during supplementation were significantly different at weeks 4, 8, and 12 at 1.25, 1.5, and 1.95 times the MED, respectively. Compared with the control values, the  $\Delta a$  values during supplementation were somewhat lower in group 2 than in group 1, indicating that vitamin E may enhance the protective effect of treatment with  $\beta$ -carotene. However, this difference was not significant.

No significant differences in protection against UV light-induced erythema were detected at different doses (from 1 to 1.95 the MED). The degree of protection was similar at 1.5 and 1.95 times the MED in group 1 and at 1.25, 1.5, and 1.95 times the MED in group 2. Approximate sun-protection factors of 2.4 in group 1 and 3.0 in group 2 were estimated after 12 wk of supplementation.

## DISCUSSION

We investigated the protective effects of a carotenoid supplement (25 mg total carotenoids) and a carotenoid supplement plus vitamin E (335 mg *RRR*- $\alpha$ -tocopherol) on the development of erythema when the skin was irradiated with a solar light simulator. The compounds were given daily for 12 wk. The major carotenoid in the supplement was  $\beta$ -carotene, which increased in serum and skin during the supplementation period. Compared with the control, ingestion of carotenoids or carotenoids plus vitamin E was associated with less erythema formation. Although not significant, erythema formation was less pronounced in group 2 than in group 1. Thus, vitamin E may provide some additional protection compared with carotenoid supplementation alone; this

TABLE 1

Serum and  $\beta$ -carotene, skin carotenoids, serum tocopherol, and  $\Delta a$  values in volunteers supplemented with carotenoids or carotenoids plus  $\alpha$ -tocopherol<sup>1</sup>

Time of supplementation and dose	Serum $\beta$ -carotene		Skin carotenoids <sup>2</sup>		Serum $\alpha$ -tocopherol		Control $\Delta a$ value <sup>3</sup>		Supplementation $\Delta a$ value	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
	$\mu$ mol/L		nmol/g		$\mu$ mol/L					
0 wk, 1.00 MED	0.54 $\pm$ 0.33	0.69 $\pm$ 0.61	0.12 $\pm$ 0.12	0.17 $\pm$ 0.21	38.6 $\pm$ 10.0	42.0 $\pm$ 10.3	4.8 $\pm$ 2.1	4.2 $\pm$ 2.9	5.1 $\pm$ 0.9	2.8 $\pm$ 1.4
4 wk, 1.25 MED	2.75 $\pm$ 1.88	2.21 $\pm$ 1.07	0.24 $\pm$ 0.13	0.38 $\pm$ 0.30	41.9 $\pm$ 12.0	77.8 $\pm$ 21.2	7.4 $\pm$ 1.9	7.7 $\pm$ 2.5	6.5 $\pm$ 1.9	4.0 $\pm$ 1.9 <sup>5</sup>
8 wk, 1.50 MED	2.69 $\pm$ 1.71	2.48 $\pm$ 1.07	0.27 $\pm$ 0.23	0.25 $\pm$ 0.20	42.2 $\pm$ 8.1	74.9 $\pm$ 21.3	9.2 $\pm$ 1.9	9.5 $\pm$ 2.0	6.5 $\pm$ 1.8 <sup>5</sup>	5.4 $\pm$ 2.0 <sup>5</sup>
12 wk, 1.95 MED	2.92 $\pm$ 2.12	2.59 $\pm$ 1.31	0.32 $\pm$ 0.32	0.24 $\pm$ 0.24	42.7 $\pm$ 6.8	75.6 $\pm$ 22.1	10.7 $\pm$ 3.3	11.0 $\pm$ 1.8	7.0 $\pm$ 2.9 <sup>6</sup>	7.5 $\pm$ 2.1 <sup>5</sup>
14 wk, 1.25 MED <sup>4</sup>	1.67 $\pm$ 1.12	1.61 $\pm$ 1.11	0.21 $\pm$ 0.21	0.29 $\pm$ 0.21	40.9 $\pm$ 6.4	47.9 $\pm$ 11.6	7.4 $\pm$ 1.9	7.7 $\pm$ 2.5	6.0 $\pm$ 2.1	6.1 $\pm$ 1.6

<sup>1</sup> $\bar{x} \pm$  SD. Group 1 (*n* = 8) received 25 mg carotenoids/d for 12 wk; group 2 (*n* = 9) received 25 mg carotenoids/d plus 335 mg (500 IU) *RRR*- $\alpha$ -tocopherol/d for 12 wk.

<sup>2</sup>Determined by reflection spectroscopy at the inside of the arm.

<sup>3</sup> $\Delta a$  value = *a* value 24 h after irradiation - *a* value before irradiation.

<sup>4</sup>Data were obtained 2 wk after cessation of supplementation; control values were obtained before supplementation.


<sup>5,6</sup>Significantly different from control (Student's *t* test): <sup>5</sup>*P* < 0.01, <sup>6</sup>*P* < 0.05.

additional protection may be associated with additive or synergistic effects of the lipophilic antioxidants.

The observed protective effects of carotenoids in this study are consistent with data reported by Gollnick et al (17), who found that the development of erythema induced by natural sunlight was lower with  $\beta$ -carotene supplementation (30 mg/d for 10 wk). A slight but significant protective effect of oral  $\beta$ -carotene supplements against erythema was also reported by Mathews-Roth (21). High doses of  $\beta$ -carotene (180 mg/d) were administered for 10 wk to volunteers who were exposed to natural sunlight for  $\leq 2$  h. The protective effect was attributed to an increase in the MED after supplementation. However, as mentioned previously, no effects were reported in another study in which 90 mg  $\beta$ -carotene/d was administered for 3 wk (16). Supplementation with  $\beta$ -carotene provided no clinically or histologically detectable protection when skin was irradiated with 3 times the MED to provoke a sunburn-type reaction. The authors concluded that  $\beta$ -carotene supplementation is unlikely to modify the severity of cutaneous photodamage.

Because of differences in the dosage and duration of  $\beta$ -carotene supplementation and in UV exposure, it is difficult to directly compare the studies and explain their outcomes. In 3 of the 4 studies, serum and skin carotenoid concentrations were measured. Baseline serum  $\beta$ -carotene concentrations were comparable and in a range that would be expected for subjects not taking supplements. The concentrations increased with supplementation; in the study that showed no protective effects, concentrations were even higher after 3 wk ( $\approx 5 \mu\text{mol/L}$ ) than they were in the present study ( $3 \mu\text{mol/L}$ ) after 12 wk of supplementation.

A comparison of skin carotenoid levels was difficult because concentrations were measured in different areas of the body, and different skin layers were included. Reflection spectroscopy provides spectroscopic data from surfaces of skin, which includes epidermis and dermis. The carotenoid levels measured in the present study are similar to those found in studies in which skin free of subcutaneous fat was analyzed. In facial skin, mean  $\beta$ -carotene levels of  $\approx 0.1$ – $0.2 \text{ nmol/g}$  wet tissue were found (22). The levels appear to be much higher when subcutaneous fat is included in the sample, as in the study in which no protective effect was found. The level of  $\approx 4 \text{ nmol/g}$  in one study greatly exceeded the levels measured only for dermis and epidermis (16). Thus, there is still some doubt about the levels of carotenoids at the target sites and further research is necessary to measure the time dependency of carotenoid uptake and turnover in the skin.

Duration of supplementation might be an important factor, probably more important than dosage. In the studies that showed protective effects, the duration of  $\beta$ -carotene supplementation was  $\geq 10$  wk, whereas in the study that showed no effects, the duration of supplementation was only 3 wk. Our data showed that supplementation with carotenoids or a combination of carotenoids and vitamin E for 12 wk at dosages exceeding dietary intakes of these antioxidants increased the basal protection of skin against erythema. 

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